Protocol Addenda

Number	Date Approved
713A	7-24-01
713B	10-16-01
713C	1-9-02
713D	3-12-02

(maximum of 4 addenda during each 3 year period)

713E	6-12-02
713F	2-28-03
713G	4-28-03

Protocol # 713
Date Submitted 12-21-00 Rev 3/16
Date CHUA Approved 3-30-01
USDA Pain Category D
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PROTOCOL FOR CARE AND USE OF LABORATORY ANIMALS
COMMITTEE FOR THE HUMANE USE OF ANIMALS (CHUA)
SUNY UPSTATE MEDICAL UNIVERSITY

THIS REVIEW IS FEDERALLY MANDATED FOR THE PURPOSE OF DETERMINING COMPLIANCE WITH EXISTING GUIDELINES AND/OR REGULATIONS FOR REASONABLE AND PROPER USE OF LIVING ANIMALS
PROTOCOLS MUST BE TYPEWRITTEN

Principal Investigator	Degree	Dept/Div.	Bldg/Room #	Phone	Home Phone	E-mail
Michael M. Meguid	M.D., Ph.D.	Surgery	8708 UH	4-6277	682-2580	meguidm@upstate.edu

Title of Protocol: Gastric Banding in the Rat – Development of an Animal Model of the Lap-Band Procedure in Humans

Yes No

X ___ Does this protocol involve survival or non-survival surgery? If yes, complete Appendix I.
___ X Does this protocol involve the maintenance of a breeding colony? If yes, please complete the Breeding Colony Information Sheet (Appendix II)
___ X Does this protocol involve animal by-products from another investigator's protocol? If yes, please complete an Animal Products Form (Appendix III)
___ X Do you anticipate using expired materials in non-survival surgery? If so, sign Guideline for Use of Expired Medical Materials and include anticipated use in the protocol (Section F: Procedures).
X ___ Does this protocol involve controlled substances? e.g. Ketamine, Buprenorphine, Somlethal, Na Pentobarbital, Pentothal.
If yes, please indicate in Section F the person responsible for controlled substances.
___ X Does this protocol involve prolonged restraint of animals? If yes, please justify the use of prolonged restraint in Section F.
___ X Does this protocol involve food or water restriction of the species used? If yes, please justify the use of food or water restriction for this project in Section F.
___ X Does this protocol involve collaborative research wherein any of the animal studies are being conducted at another institution? If yes, please attach the following: (1) Institutional Animal Care and Use Committee (IACUC) approval letter (2) the Instituional Assurance Number (3) a copy of the protocol.
___ X Does this protocol involve the *in vivo* use of recombinant DNA and/or infectious agents? If yes, approval from IBC must be obtained and a copy of the approval letter and application provided . Any protocol which includes the use of recombinant DNA and/or infectious agents must have prior approval from the Institutional Biosafety Committee (4-5160).
___ X Does this protocol involve the *in vivo* use of radioactive materials? If yes, approval from the Radiation Safety Office (X46510) must be obtained and a copy of the approval letter and application provided . Please provide License Number_____.
___ X Does this protocol involve the *in vivo* use of carcinogens, toxins or mutagens? If yes, approval from Environmental Health & Safety (45782) must be obtained and a copy of the approval letter and application provided .
___ X Does this protocol involve animals previously used on another project? If yes, provide investigator name and CHUA Number. PI Name:_____CHUA #_____

(Revised January 2000)

Name	Degree	Dept/Div.	Work Phone	Home Phone	email*	Pager
Y. Qi	M.D.	Surgery	464-6309		qiy	

Has this individual attended the Introductory Training Session or viewed the training video? Yes X No
Qualifications: Y. Qi, MD: Dr Qi is a research fellow currently involved in research using rodents at SUNY Upstate Medical University, Syracuse, Department of Surgery. He has been involved in research using rats for last 4 years and is therefore knowledgeable in regards to major aspects of their care, including correct techniques for aseptic rodent surgery, animal anesthesia, and euthanasia in regards to our experimental protocols.

Name	Degree	Dept/Div.	Work Phone	Home Phone	email*	Pager
Robert Quinn	DVM	Director-DLAR	4-6563	682-4996	Quinnr	467-9991

Has this individual attended the Introductory Training Session or viewed the training video? **Yes** No
Qualification for the proposed study:
Director of Animal Resources. 9 years experience using animals in research, including rodent surgery.

If unfamiliar with this species/procedure, please indicate person responsible for training:

B. PROCUREMENT & HOUSING SPECIFICATIONS FOR ANIMALS IN THIS PROTOCOL:

	Species 1	Species 2
Species:	(a) Rat	(b) Rat
Strain or Breed:	(a) Sprague-Dawley	(b) Zucker (obese)
Preferred Source (if any)	(a) Harlan or Charles River	(b) same
Sex:	(a) Male	(b) male
Age or Weight:	(a) 220-240 g.	(b) 300 g.
Total # of animals to be used per year:	(a) Year 1: 24	(b) Year 1: 16
	(a) Year 2:	(b) Year 2:
	(a) Year 3:	(b) Year 3:
	(a) Total: 24	(b) Total: 16
Average daily census:	(a) 8	(b) 16
Average duration of housing:	(a) 3 months	(b) 3 months

Are there any special housing requirements? Yes X No
Please list special housing requirements: ACREM Cages

If the animals are to be removed from the DLAR vivarium, please give the specific location of the laboratory and how long the animals will be kept in the laboratory:

Location of laboratory (Bldg. & Room No.)	(a) 8805 UH	(b)
Duration:	(a) 1-2 Hours	(b)

List the procedures (from Section F) that will be performed outside of the vivarium:

Gastric banding surgery
Euthanasia

11. Meguid MM, Fetissov SO, Varma M, Sato T, Zhang L, Laviano A and Rossi-Fannelli F. Hypothalamic dopamine and serotonin in the regulation of food intake. Nutrition 16:843-857, 2000

12. Meguid, M.M., Kawashima, Y., Campos, A.C.L., Gelling, P., Hill, T.W., Chen, T-Y, Hitch, D.C., Mueller, W.J., Hammond, WG: Automated computerized rat eater meter: Description and application. Physiol. Behav. 48:759-763, 1990

ii. What value or potential contributions to biology or medicine may come from this work?

The central mechanisms of appetite control are not well understood. Obesity contributes to a number of severe disease states that affect a large proportion of the population. A model such as this would allow the study of how central neurobiologic processes change when an obese individual with increased appetite becomes an obese individual with decreased appetite. This type of model should allow for a much better understanding of central mechanisms involved in appetite control, which could be used to develop pharmacological or other methods for central appetite control within the general population.

iii. Please give detailed reasons why animals must be used.

Appetite is affected by a complex interaction between the central nervous system, local gastric reflexes, hormonal affects, mechanical affects and numerous other processes. This type of system is far to complex to allow modeling *in vitro* or computer simulation. It is this complex nature that has made the understanding and control of appetite such an elusive problem, and therefore exemplifying the need for this type of animal model.

iv. Please give detailed reasons why this species must be used?

There is a tremendous volume of literature utilizing the rat as a model of appetite control *(for a review, see reference #11, Meguid MM, Fetissov SO, Varma M, Sato T, Zhang L, Laviano A and Rossi-Fannelli F. Hypothalamic dopamine and serotonin in the regulation of food intake. Nutrition 16:843-857, 2000).* We have used this model for numerous studies in the past and therefore have significant experience and previous data with which to make comparisons with this new model. It is the smallest mammal available that will allow the future in vivo preparations to study neurotransmitter changes.

v. Are you using the fewest number of animals possible? Please explain*. Include the number of groups with the "n" value for animals clearly outlined.

Extensive previous experience has determined that a minimum of 8 animals per group is required for statistical determination of differences in eating patterns as measured by the ACREM units.

*(A guideline that is sometimes appropriate is that the sample size should be adequate to detect reliably a 10% difference in treatment means. If not statistically justifiable, please justify the number of animals to be used *or* indicate this is a pilot study designed to demonstrate the amount of variability expected in the data.)

E. **PROCEDURES: Describe <u>ALL</u> experimental procedures involving animals in your protocol.** In the case of multiple experimental procedures a flow diagram accompanying the narrative description is often very useful.

and approximately 5 mm from the esophagus. The tubing is cut to the exact length and the ends are sutured together with 5-0 nylon to make an encircling loop. The loop is then tacked in place with three sutures which encircle the tubing and incorporate the submucosa of the stomach. The wound is closed in 3 layers (vicryl internal, nylon external), the oral cannula is deflated and removed and the animal is allowed to recover from anesthesia. Buprenorphine (0.05 mg/kg) will be administered twice daily for at least 48 hours post-op.

Criteria for Study Termination:

Since this is a new model and involves expected weight loss, the following criteria will be followed to determine the termination point of the study for any individual animal:

Weight loss in excess of 30% of baseline (as measured just prior to surgery).
Complete anorexia of 3 days duration.
Any animal that is moribund or in obvious physical distress.
Any other situation deemed necessary in consultation with the attending veterinarian.

Address the following in this section if applicable:

- **Expired Medical Materials**
  Outline any anticipated use of expired medical materials.

NA

- **Controlled Substances**
  Person responsible: Dr. Lihua Zhang
  Are Controlled Substances kept in a double locked cabinet and records of use maintained? X Yes No

- **Food & Water Restriction**
  Justify the use of food or water restriction (if applicable).

- **Prolonged Restraint**
  Justify the use of prolonged restraint (if applicable).

F. EUTHANASIA: Describe the euthanasia method(s) to be used, including the dosage and route of administration of any drugs to be used. All euthanasia methods must comply with the *1993 Report of the AVMA Panel on Euthanasia* (J.A.V.M.A. Vol. 202, No. 2, pg 229-249) or scientific justification must be provided for "conditionally approved methods." **It is essential to ensure that death has definitely occurred before the body of any animal is discarded.**

Rats will be euthanized by decapitation under anesthesia to harvest hypothalamic nuclei for measurement of monoamines, NPY and leptin. Using data from our previous protocol for comparison purposes to test the validity of our postulate that these factors are involved in the mechanism for the control of food intake, as studied in our cancer and anorexia and obesity protocols. *The gastric banding will then be inspected for integrity and consistency.*

G. GUIDELINES: If your protocol involves any of the following procedures, please sign and submit the appropriate forms:

H. PAIN and/or DISTRESS LEVEL (Please read and sign the following page prior to completing this section.)

Indicate which category(s) your protocol comes under per USDA definitions:

_____Category C: Pain and/or distress no greater than an injection.
__X__Category D: Pain and/or distress fully alleviated with analgesics/anesthetics.
_____Category E*: Pain and/or distress not fully alleviated with analgesics/anesthetics.

For protocols in *either* Categories D or E the following requirements must be addressed:

(a) The Principal Investigator must provide a list of the specific anesthetics and analgesics along with their dose, frequency and route of administration.

Anesthesia: Halothane (1-3%, inhalation to effect) or a mixture of ketamine, xylazine and acepromazine (150:30:5 mg/ml) administered at 0.5-0.7 ml/kg IP.

Analgesia: Buprenorphine 0.05 mg/kg SQ twice daily for at least 48 hours after surgery.

(b) The Principal Investigator must provide a written narrative of the methods and sources used to determine that alternatives were not available. The minimal written narrative as stated in USDA Policy #12 should include: (a) the databases searched or other sources consulted, (b) the date of the search and (c) the years covered by the search, (d) the key words and/or search strategy used by the Principal Investigator when considering alternative. Include descriptions of other methods and sources used to determine that no alternatives were available to painful or distressful procedures.

The narrative should be such that the CHUA can readily assess whether the search was sufficiently thorough. Reduction, replacement and refinement (the three R's) must be addressed, not just animal replacement. (The Three R's: **Reduction** in number, **Replacement** of animals, and **Refinement** of technique to reduce pain and distress.)

Possible databases include, but not are limited to: MEDLINE, TOXNET, AIDSLINE, CANCERLIT, CURRENT RESEARCH INFORMATION SERVICE (e.g. Current Contents, Index Medicus) and ANIMAL WELFARE INFORMATION CENTER (National Agricultural Library, 301-504-5755 or 5756).

Medline was searched on 12/19/00 using the following terms: Bariatric surgery, models, animal, alternatives, gastric banding. No alternatives were identified.

* (*Please note*: The Principal Investigator must present a written rationale to the CHUA and may be requested to attend a Panel meeting to discuss the proposed research when a protocol falls into Category E)

SIGNATURE PAGE

I certify that the animals used in this study will be cared for and used in accordance with regulations and standards as promulgated by the New York State Health Department, the Federal Animal Welfare Act, the Public Health Service and the Department of Laboratory Animal Resources Policies and Procedures of the SUNY Upstate Medical University.

Moreover, I certify:

1. that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research;
2. that analgesic, anesthetic and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals;
3. that I have considered alternatives to painful procedures;
4. that the use of *in vitro* or other alternative techniques to the use of animals has been considered;
5. that I have concluded that the species, numbers and procedures to be used are the most appropriate for the proposed study;
6. that the proposed research does not unnecessarily duplicate previously published experiments.
7. that I understand that any animal displaying signs of post-procedural pain and/or distress that is not resolved with standard veterinary therapies will be euthanized.
8. any individuals involved in this project will be properly trained prior to beginning work on animals.

Michael Neginsk MD PhD

Signature of Principal Investigator Michael N. Niginski, MD PhD **Date:** 3/14/01

Signature of Department Chairperson KR **Date:** 3/16/01
(required if animals are to be used in teaching *or* if the protocol is not part of a sponsored project)

Following CHUA approval of your protocol, please contact DLAR to receive clarification of animal room assignments and space <u>BEFORE</u> submitting animal purchase requisitions.
Thank you.

APPROVAL IS EFFECTIVE FOR 1 YEAR AND RENEWAL MUST BE SECURED BEFORE THE END OF THE APPROVAL MONTH.

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CHUA Chairperson's Signature **Approval Date:** 4-6-01

Veterinarian's Signature **Approval Date:** 3/30/01

Can the surgery be performed in DLAR surgical facilities? Yes X No
If not, please provide justification for the location chosen:

Rodent surgical space already equipped and available in investigator's laboratory.

Post Operative Care:

Post operative care in DLAR (4th floor, WH or 7th floor, UH) or indicate location of post operative care room and name of person monitoring recovery.	DLAR?__X_Yes ____No or Location: Name: Drs. ~~Zhang~~ Xu and Quinn Frequency? At least daily By Whom?	DLAR?____Yes ____No or Location: Name: Drs. ~~Zhang~~ Xu and Quinn Frequency? At least daily By Whom?
Analgesia. List drugs, dose, route of administration and frequency.	Buprenorphine 0.05 mg/kg IP Twice daily for at least 48 hr.	Buprenorphine 0.05 mg/kg IP Twice daily for at least 48 hr.
Treatment. If any medications are given, please list agent(s), dose and route of administration.		
Other (suture removal, etc.)	Sutures removed at 7-10 days.	Sutures removed at 7-10 days.

** Please address the following items:

(a) Describe pre-operative procedures, including fasting, pre-medication and preparation of surgical site.

Animals will be fasted for approx. 18 hours prior surgery to minimize gastric contents.

(b) Approximate length of surgical procedure.

30 minutes.

(c) Provide a detailed description of the surgical procedure, including site of incision, operative manipulations, method of closure, suture material, etc.

See section E.

(d) Describe any post-operative complication(s) that may be anticipated and the steps planned to monitor and treat the animal.

Postoperative complications may include infection or anorexia. Infection will be treated with the appropriate antibiotic as determined by a culture and sensitivity. If treatment is ineffective or if the animal is completely anorexic, it will be euthanized. For other criteria, see section E.